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The Genetics of Some Polymorphic Forms of the Butterflies *Heliconius melpomene* (Linnaeus) and *H. erato* (Linnaeus).

II. The Hybridization of Subspecies of *H. melpomene* from Surinam and Trinidad.[1,2]

(Plate-figures 1-37; Text-figures 1-4; Tables 1-10)

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To investigate the genetics of mimetic patterns in *H. melpomene,* which mimics its own relatives in the genus *Heliconius,* crosses were performed between geographical races from Trinidad and central Suriname (South America). The differences between the color patterns, so distinct as to be classed as separate species by some authors, are controlled by four major loci (designated *B, D, N,* and *F*), all showing complete dominance except for *N*. The only linkage is between *B* and *D,* with crossovers less than 16 percent. Some of the F_2 hybrids showed patterns not found in the parental subspecies, but known in other races or in related species. These were in part controlled by the *N* locus. There is evidence of other loci, in addition to the four major ones, affecting the pattern. The race from eastern Amazonia differs from that in central Suriname by an allele at the *D* locus. In northern Suriname, these three races hybridize naturally, producing a very elaborate polymorphism. Previous workers have bred butterflies from this polymorphic area and have therefore only partly solved the genetics of the race differences. The present study, tabulating 54 crosses (over 1,000 butterflies), has probably detected most of the major genes differentiating the three subspecies.

INTRODUCTION

THE NEOTROPICAL BUTTERFLY *Heliconius melpomene* is interesting because of its close mimicry of its relative *Heliconius erato* (illustrated by Turner, 1970), and because it exhibits elaborate polymorphisms in certain parts of its range. Research into this Müllerian mimicry and polymorphism is likely to be particularly profitable, as the New York Zoological Society has conducted intensive research into many aspects of the biology of *Heliconius* and related genera (for a review of certain aspects, see Turner, 1971a), giving exceptional opportunities for integrated research.

This paper describes a thorough investigation of the genetics of the polymorphism of *melpomene* in the Guianas. The main aim was to solve certain problems posed by previous breeding experiments with this species (Turner and Crane, 1962; Sheppard, 1963); it is thought that most of the genetic factors have now been identified. Sheppard (1963) found that in this species, different genes can produce identical phenotypes; for this reason the new broods will be analyzed first, and the results will then be compared with those of the previous workers.

Turner and Crane (1962) and Sheppard (1963) obtained their stocks from the monomorphic populations of the species in Trinidad (Text-fig. 1c) and from Moengo in Surinam (Text-fig. 2) where there is a polymorphic popu-

[1] A contribution of the New York Zoological Society's Department of Tropical Research.

[2] Dedicated to Professor E. B. Ford on his seventieth birthday, in honor of his contributions to the study of butterflies and their genetics.

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lation consisting mostly of butterflies like those in Trinidad, with a small percentage of "mutant" forms; frequencies are given by Sheppard (1963). The present broods are different from these in one most important way: the Surinam stock was obtained at Brokopondo (Text-fig. 2), where *melpomene* has a population consisting mostly of butterflies with the pattern shown in text-fig. 1b, and very unlike individuals from Trinidad. The stocks were thus started with lines differing simultaneously at several loci.

It will be shown elsewhere (Turner, 1971b) that the population at Brokopondo is on the edge of a subspecies of *melpomene* (*H. melpomene meriana* Turner), found in the interior of the Guianas. The present breeding experiments are tantamount to hybridization of two subspecies of *melpomene,* although, rather conveniently, the slight degree of polymorphism of the Brokopondo population resulted in a few segregating broods produced by wild-caught females. This considerably speeded the work.

This paper replaces the paper originally projected as the second in this series (and cited by Turner and Crane, 1962), as the matter to be discussed (the inheritance of various yellow markings) is much more clearly understood from the new broods reported here.

Methods

The parent butterflies which founded the Surinam stock were captured September 22, 1964, in disturbed rain-forest near Brokopondo, which lies almost as far into the interior of Surinam as can be reached by road. They were kept overnight in a portable gauze cage, fed forcibly on sucrose solution, and flown next day to Trinidad. Trinidadian parent butterflies were reared from early stages found in northern Trinidad.

The methods of mating and rearing the insects in Trinidad have already been described (Turner and Crane, 1962). In December 1964, the larvae (with leaves of the food plant) and the pupae were packed in capped, plastic tubes, and the imagines were placed in paper photonegative envelopes, with a wad of cotton-wool soaked in sucrose solution. The whole stock was placed in an insulated picnic hamper, and carried to Britain in the passenger cabin of a jet airliner. Despite the decompression and, on arrival, a train journey of several hours through mid-winter Britain, the stock reached Liverpool with all the insects alive.

In Liverpool the larvae were reared in plastic boxes, several to a box, or in cloth sleeves on food plants growing in greenhouses. Greenhouses were used for mating and for housing laying females. Butterflies were fed from dishes of honey mixed with water. Only four greenhouses were available, including one used for a group of mixed broods, which were maintained as a general reservoir of genes without full records. As a female requires a whole small greenhouse to fly in, we could keep only three fertile females at a time, and as mortality of adults and larvae was much higher than it had been in Trinidad, broods from Liverpool were smaller than those from Trinidad. Despite the less than optimum conditions, stocks were maintained until May 1965.

In Trinidad, *Passiflora laurifolia* and *P. serrato-digitata* were used as food plants; in Liverpool, many of the larvae were reared on the horticultural hybrid *P. allardi,* which they readily accepted.

Specimens are preserved in translucent envelopes (see Turner and Crane, 1962) and stored in a filing system. With the large number of cages used in Trinidad, breeding strategy was

Text-figure 1. Three forms of *Heliconius melpomene:* (a) the Amazonian *H. m. thelxiope* (Hübner); (b) the Surinamian *H. m. meriana* Turner; (c) the Trinidadian and Venezuelan *H. m. melpomene* (Linnaeus). About 0.8 times natural size.

comparatively easy, and almost every butterfly has been kept. In Liverpool it was necessary to release a large number of butterflies into the greenhouse containing the general stock, and because of this, and the loss of butterflies which died naturally behind the elaborate fittings in the greenhouse, not all the specimens hatched in Liverpool are preserved.

The results are shown in Tables 1-7. A "?" indicates a wild butterfly, mated to one of the Surinam females, and never seen; parental butterflies are described by their brood of origin (with "Surinam" and "Trinidad" for wild butterflies, and "Liverpool" for butterflies from the general gene-reservoir already mentioned) and phenotype; the mating numbers merely refer to the original brood books and are not used in the text; a small superscript letter (e.g.[a]) identifies a butterfly which was the parent of more than one brood; § indicates that several males were placed with one female, and that the butterfly listed is the most probable mate, in view of the progeny obtained. Phenotype formulae are described in the next section; brackets indicate a doubtful phenotype.

The system of individual rearing and labeling used in Trinidad resulted in extremely few errors. Only two such mistakes appeared: on S^{F}Dr butterfly in brood 17 and a W^{F}dr in brood 11; clearly the labels had simply been reversed, and the butterflies have been placed in their correct brood in the tables.

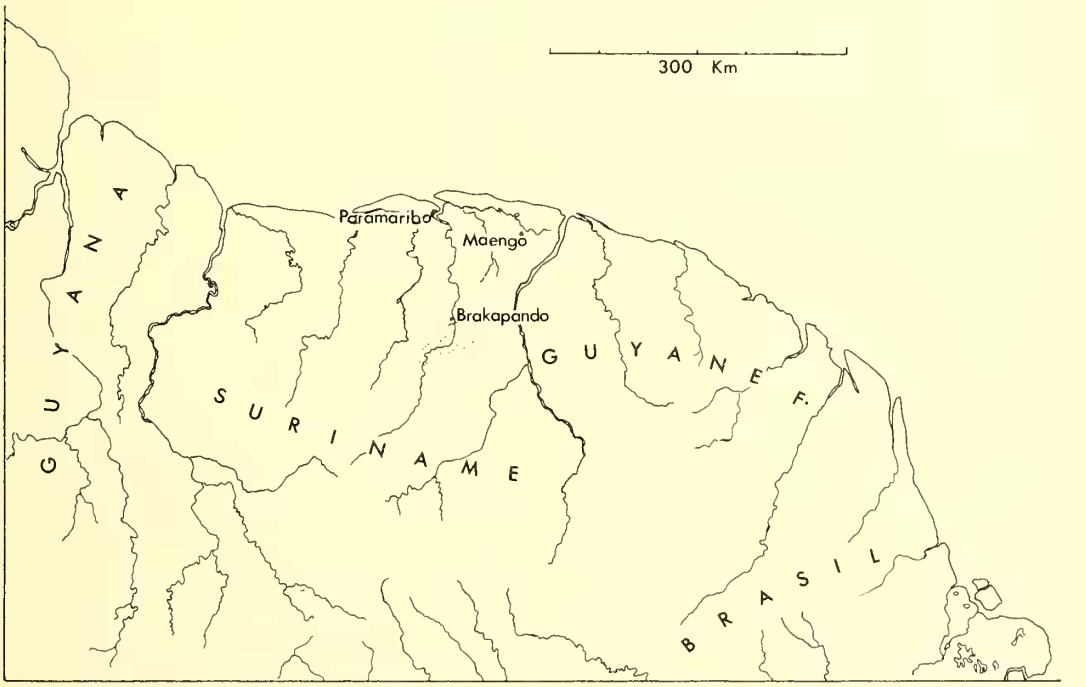
Major Genes

Inheritance of dennis and radiate

The pattern known as "dennis" (Dr) consists of extensive red patches covering the basal half of the forewings, and a small red triangle at the base of the hindwings. The radiate pattern (DR) is the same as dennis, but has in addition about six red rays between the veins on the hindwings. A butterfly lacking these red marks is called plain (dr). The plates and text-fig. 1 show examples.

Trinidadian butterflies are always plain; all the butterflies from Brokopondo were dennis, but one female produced a brood that segregated dennis and radiate in equal numbers (Table 1, brood 3).

Dennis is produced by a single gene dominant to plain; the F_1 hybrids between Surinam (dennis) and Trinidad (plain) are all dennis (Table 1, broods 5-7), the F_2 segregates 64 dennis to 27 plain (Table 4, total), and the backcross to Trinidad segregates 64 dennis to 69 plain (Table 3, total). Radiate is dominant to plain, and likewise dominant or epistatic to dennis. Thus Surinam radiate butterflies mated to plain Trinidad butterflies yielded broods consisting equally of radiate and dennis (ratio 17 DR to 21 Dr),



Text-figure 2. The Guianas, showing Moengo and Brokopondo, where polymorphic forms of *melpomene* have been obtained for breeding experiments.

Table 1. Offspring of Wild Surinam Females, and F_1 Surinam × Trinidad Hybrids

Brood	Mating	Parents ♀	Parents ♂		Radiate (DR) Y^F	Radiate (DR) TS^F	Dennis (Dr) Y^F	Dennis (Dr) TY^F	Dennis (Dr) S^F	Dennis (Dr) TS^F	Total
1	M5	Surinam	Surinam	♂	0	0	10	3	5	7	47
		TY^FDr	?	♀	0	0	6	5	6	5	
2	M6	Surinam	Surinam	♂	0	0	17	0	11	0	57
		S^FDr	?	♀	0	0	15	0	14	0	
3	M7	Surinam[a]	Surinam	♂	26	0	20	0	0	0	80
		Y^FDr	?	♀	16	0	18	0	0	0	
4	M9	Surinam	Surinam	♂	0	0	16	0	0	0	23
		Y^FDr	?	♀	0	0	7	0	0	0	
5	M13	Trinidad	Surinam	♂	0	0	0	0	0	26	56
		W^fdr	Y^FDr	♀	0	0	0	0	0	30	
6	M14	Trinidad	Surinam	♂	0	0	0	0	0	22	51
		W^fdr	Y^FDr	♀	0	0	0	0	0	29	
7	M16	Trinidad	Surinam[d]	♂	0	0	0	0	0	9	21
		W^fdr	Y^FDr	♀	0	0	0	0	0	12	
8	M22	3	Trinidad[b]	♂	0	4	0	0	0	4	20
		Y^FDR	W^fdr	♀	0	4	0	0	0	8	
9	M23	3	Trinidad	♂	0	6	0	0	0	4	19
		Y^FDR	W^fdr	♀	0	3	0	0	0	6	

showing that the radiate pattern is dominant to plain (which is not expressed in the hybrid), and also to dennis (which must have been carried, but not expressed, by the radiate parent) (Table 1, broods 8-9). Strictly we should say that radiate is "not recessive," rather than "dominant" as a radiate homozygote has not for certain been produced; brood 50 (Table 7) is a cross between two radiate butterflies, but the 6 radiate offspring are too few to contain, with reasonable probability, a homozygote. But it is likely that the radiate homozygote is in fact like the heterozygote, and the gene completely dominant, as the subspecies *thelxiope* from Pará is monomorphic, and so presumably homozygous, for this pattern.

Allelism of dennis and radiate

As the radiate pattern contains the dennis pattern, it seems fairly safe to assume that radiate and dennis are alleles at the same locus. However they might well be produced by two independent loci, which both exploited the same developmental pathway. Broods 8, 18, 21, and 34 show that they are in fact alleles at a single locus. A Surinam radiate butterfly was mated to a plain from Trinidad. The progeny (Table 1, brood 8) show that the radiate parent was carrying dennis but not plain. Therefore if radiate and dennis were at different loci, with radiate epistatic to dennis, the radiate progeny in brood 8 should be carrying both dennis and plain; on mating to a plain butterfly, they should give radiate, dennis, and plain in the ratio 2 : 1 : 1. On the other hand, if radiate and dennis are allelic, the radiate progeny in brood 8 can carry only radiate and plain, and on crossing to a plain butterfly will give radiate and plain in the ratio 1 : 1, but no dennis.

Two of the radiate progeny, mated to plain butterflies produced, in broods 18, 21, and 34 (Tables 3 and 5), 17 butterflies which were either radiate or plain; there were no dennis. The appropriate statistical test is to calculate the probability that no dennis butterflies will appear in a brood of 17 individuals when one quarter of the brood are expected to be dennis. The test is one-sided; for if the whole brood was by chance dennis, no statistical test would be needed. By the binomial theorem, the probability of this happening is $(0.75)^{17} = 0.0075$, which is highly significant. It is reasonable, therefore, to conclude that radiate and dennis are not carried at separate loci, but are allelic.

The above test does not of course rule out the possibility that radiate and dennis are at two loci which are linked rather closely, but as they are certainly on the same chromosome, it is simpler to regard them as alleles. The patterns radiate, dennis, and plain can be thought of as controlled by three alleles D^R, D, and d. An alternative hypothesis is that the patterns are controlled by two closely linked loci, one (D) producing the dennis pattern, the other (R) adding the rays. The patterns would then be con-

TEXT-FIGURE 3. The three main forms of the Ecuadorian species *Heliconius timareta* (Hewitson). About ¾ times natural size.

trolled by three chromosomes *DR*, *Dr*, and *dr*. This theory is supported by the closely related species *Heliconius timareta* from the eastern slopes of the Andes in Ecuador, one of whose three main forms has rays without the dennis pattern (Text-fig. 3). If dennis and ray are indeed closely linked loci, then the recombination value is very low, as no individual showing ray without dennis has ever been found among the thousands of specimens exported from the polymorphic populations of Guyane (Joicey and Kaye, 1917).

Inheritance of color of bands

The band on the forewing may be of the following types, designated by letters in the tables:

Y or "yellow". A group of firm pale yellow marks outside and inside the cell; the scales within the marks are entirely yellow and not mixed with black (Plate-figs. 1-4).

TY or "thin red with yellow". Like "yellow," but having a thin red band round the outside of the outermost yellow marks; the width of the red varies, but is usually no more than a series of red edgings to the outermost and most anterior yellow spots (Plate-figs. 5-9).

S or "dusky yellow". A group of pale yellow green marks in the same positions as those of Y; the marks tend to be smaller than in Y, and the yellow patch in the cell is often reduced or absent. The yellow green color is produced by a mixture of black and yellow scales (Plate-figs. 10-13).

TS or "thin red with dusky yellow". Like S, but with a thin red band exterior to the yellow marks; this red band varies in width, but is normally much wider than it is in TY, being a definite bar of red curving through the whole length of the area occupied by the band (Plate-figs. 14-19).

TABLE 2. FIRST GENERATION BACKCROSSES TO SURINAM STOCK
(See Also Brood 46 in Table 7)

BROOD	MATING	PARENTS ♀	PARENTS ♂		RADIATE (DR) Y^F	RADIATE (DR) TY^F	RADIATE (DR) S^F	RADIATE (DR) TS^F	DENNIS (Dr) Y^F	DENNIS (Dr) TY^F	DENNIS (Dr) S^F	DENNIS (Dr) TS^F	Total
10	M36	6	3	♂	0	0	0	1	0	0	0	2	5
		TS^FDr	Y^FDR	♀	1	0	0	0	0	0	0	1	
11	M49	3	5[c]	♂	0	0	2	2	3	8	6	2	42
		Y^FDR	TS^FDr	♀	0	6	1	0	1	3	6	2	
12	M50	7	Surinam[d]	♂	0	0	0	0	3	3	1	1	13
		TS^FDr	Y^FDr	♀	0	0	0	0	1	1	2	1	
13	M51	5	3[e]	♂	1	6	3	2	1	7	3	0	52
		TS^FDr	Y^FDR	♀	5	1	5	3	5	1	3	6	
14	M52	5	3[e]	♂	5	0	1	0	1	2	3	2	25
		TS^FDr	Y^FDR	♀	1	2	2	3	0	2	1	0	
15	M60	Combination		♂	1	0	1	0	0	0	0	0	3
		of M51 and M52		♀	0	0	1	0	0	0	0	0	
		Total*			14	15	16	11	11	23	22	15	127*
		Total*				56				71			127*

* Excluding brood 12.

O or "absent band". The band is virtually absent, and is represented only by a few faint green-yellow marks in the region of the cell, and a red C-shaped mark near the posterior angle of the wing (Plate-fig. 20).

W or "wide red". A broad red band covering all the area in the region of the cell; in Trinidadian butterflies and in many of the hybrids, this is covered on the underside with white (sometimes yellow) scales (Plate-figs. 21-23).

The Trinidadian butterflies were always W; all Surinamian butterflies used as parents of F_1 and backcross generations were Y; two others were S and TY, and their offspring are not listed with F_1's or backcrosses.

Early in the experiments, it became obvious that band-color was controlled by more than one locus. The TY female from Surinam (mate unknown) produced a brood (Table 1, brood 1) of Y, TY, S, and TS in roughly equal numbers, suggesting that two loci are segregating. The S female from Surinam produced a brood (Table 1, brood 2) (apparently a backcross) equally of S and Y; an S × Y cross produced a similar backcross brood (table 7, brood 39). The F_1 Surinam × Trinidad hybrids (Y × W) are always TS (Table 1, broods 5-9). An S female from brood 2 mated to a W Trinidadian male, produced approximately equal numbers of TS and W offspring (Table 7, brood 41).

From these results, and bearing in mind the findings of Turner and Crane (1962) and Sheppard (1963), I formed the hypothesis about the inheritance of band-color illustrated in text-fig. 4. Two loci, *B* and *N*, are involved; the recessive allele *b* reduces the amount of red in the band, and the semi-dominant allele N^N reduces the amount of red and increases the amount of yellow. W butterflies from Trinidad are of the genotype BBN^BN^B; Y butterflies from Surinam are bbN^NN^N; the S phenotype is produced by the heterozygote N^NN^B, and the addition or subtraction of the red T mark from the S or the Y pattern is controlled by the substitution of *B* for *b*. The phenotypes of BBN^NN^N and bbN^BN^B butterflies (top right and bottom left of text-fig. 4) were uncertain, but I guessed that they would be TY and something similar to TS (not shown in text-fig. 4).

Broods which emerged after the formation of this hypothesis confirmed it, except in one detail, the phenotype of bbN^BN^B. This genotype has been produced in two matings between butterflies known to be BbN^BN^B (Table 6, broods 35 and 37), and turns out to be "absent-band" or O (Text-fig. 4; Plate-fig. 20).

Apart from this modification all test-crosses performed conform to the hypothesis. Thus Y should be homozygous; broods 40, 42, 44, and 48 which are Y × Y matings produced 80 Y butterflies in all, and no other phenotypes (the anomalous individual in brood 50 will be discussed later). An S × S mating should produce O, S, and Y; brood 47 (Table 7) has produced

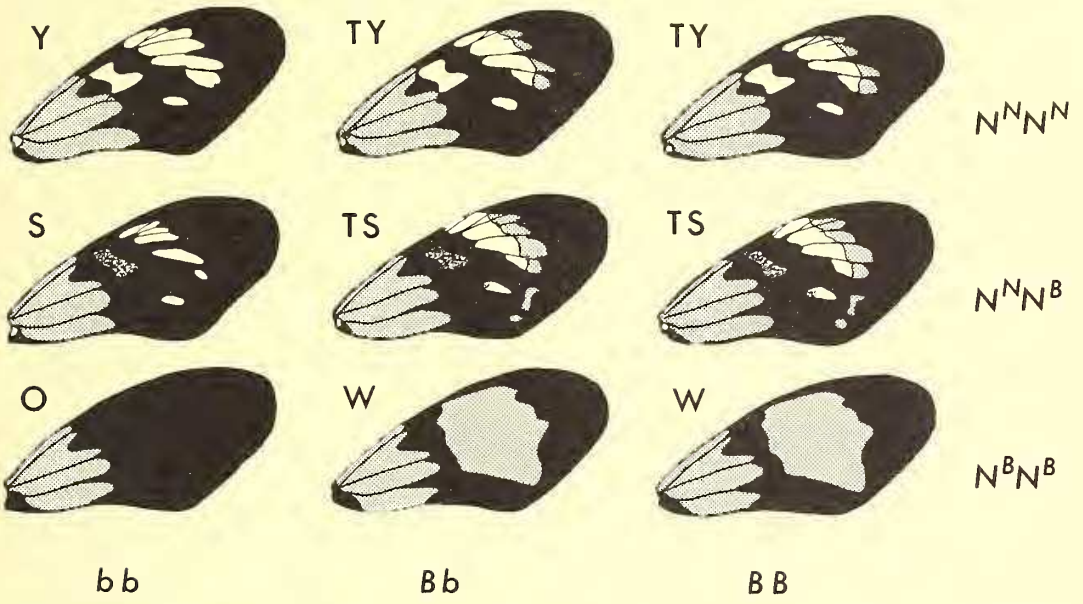
Table 3. First Generation Backcrosses to Trinidad (and a Similar Brood)

BROOD	MATING	PARENTS ♀	PARENTS ♂		DENNIS (Dr) TS^F	DENNIS (Dr) TS^f	DENNIS (Dr) W^F	DENNIS (Dr) W^f	PLAIN (dr) TS^F	PLAIN (dr) TS^f	PLAIN (dr) W^F	PLAIN (dr) W^f	Total
16	M21	Trinidad	1	♂	1	1	0	3	0	0	0	2	20
		W^fdr	TS^fDr	♀	2	1	0	2	1	4	1	2	
17	M46	Trinidad	5[c]	♂	3	3	4	7	3	6	3	3	61
		W^fdr	TS^FDr	♀	3	4	3	6	4	2	4	3	
18	M58	Trinidad	8	♂	0	0	0	0	0	0	0	0	1*
		W^fdr	TS^FDR	♀	0	0	0	0	0	0	0	0	
19	M35	6	Trinidad[b]	♂	4	0	0	0	1	4	0	3	23†
		TS^FDr	W^fdr	♀	1	1	2	0	0	2	0	4	
20	M41	5	Trinidad	♂	1	1	1	3	1	2	0	3	28
		TS^FDr	W^fdr	♀	3	0	2	2	1	2	4	2	
21	M56	8	Trinidad	♂	0	0	0	0	0	0	0	0	1‡
		TS^FDR	W^fdr	♀	0	0	0	0	0	0	0	0	
		Total			18	11	12	23	11	22	12	22	131
		Total			29		35		34*		35†		134*†‡
		Total			64				69*†				134*†‡

* 1 male, TSdr, not scored for F.

† Includes one Wdr butterfly, not scored for F or sex.

‡ 1 male, TS^FDR.



TEXT-FIGURE 4. The interaction of the *B* and *N* loci in determining the color of the band. About 0 times natural size.

S and Y, but the absence of O is not significant in a brood of only 6. A Y individual mated with a W butterfly known from its pedigree to be heterozygous *Bb* produced, as predicted, roughly equal numbers of S and TS (Table 7, brood 43). Brood 52 (Table 7) is a cross between S and TS (the bracket round the S in the table indicates that yellow marks were virtually absent); it segregates 4 TY, 12 TS, and 5 W, a satisfactory approximation to the ratio 1 : 2 : 1 expected if the TS parent was homozygous *BB* (from its pedigree it had an even chance of being homozygous).

The first generation backcrosses to Surinam (TS × Y) and to Trinidad (TS × W) also confirm the hypothesis. Those to Surinam (Table 2) gave, as expected, Y : TY : S : TS in the ratio 1 : 1 : 1 : 1 (actual numbers, including brood 46 from Table 7, are 33 : 46 : 43 : 29; $\chi^2_{(3)} = 5.2$; $P > 0.1$). Those to Trinidad (Table 3) gave TS : W in the ratio 1 : 1 (actual numbers, including brood 16, are 64 : 70). The F_2 broods, produced by sib-mating F_1 hybrid butterflies (Table 4), segregate Y : TY : S : TS : O : W in the numbers (including brood 25) 4 : 27 : 9 : 38 : 0 : 17. The expected ratio from the hypothesis as shown in text-fig. 4 is 1 : 3 : 2 : 6 : 1 : 3, or in numbers 5.9 : 17.8 : 11.9 : 35.6 : 5.9 : 17.8. This gives $\chi^2 = 12.2$ for 5 degrees of freedom, which is significant at the 5 percent level. The F_2 broods have therefore segregated all the expected phenotypes, except O, and the absence of this phenotype is mainly responsible for the significant deviation from the expected ratio. Reasons will be advanced later for thinking that the genotype bbN^BN^B may sometimes produce a phenotype very like TS; in that event the expected ratio is 1 : 3 : 2 : 7 : 3, which gives $\chi^2 = 6.4$ for 4 degrees of freedom, which is not significant. The segregation of the F_2 broods therefore indicates that the hypothesis is probably correct, but that there are some additional complications which are not yet understood.

The genotype BBN^NN^N has not been formed for certain in these broods; reasons will be given later for thinking that it does indeed produce the phenotype TY.

Inheritance of shape of bands

The variation in the red bands (W or T) has been explained fully by the loci *B* and *N*. The yellow marks in the band may be either broken up into a series of spots (Plate-figs. 1-2, 5-7, 10-11, 14-16), or joined together into a yellow patch (Plate-figs. 3-4, 8-9, 12-13, 17-20). These phenotypes are indicated in the tables by superscript letters, F for a broken band and f (fused) for a joined one. This variation is found in the phenotypes Y, TY, S, TS, and probably O (the only two individuals obtained being apparently O^f). In the phenotype W (wide band), the variation is found in the distribution of white (or yellow) scales on the underside of the band, which are evenly spread in W^f phenotypes, but

TABLE 4. F_2 SURINAM × TRINIDAD HYBRIDS
(AND A SIMILAR BROOD)
(All Parents Have Phenotype TS^FDr)

BROOD	MATING	PARENTS		DENNIS (Dr)										PLAIN (dr)										Total
				Y^F	Y^f	TY^F	TY^f	S^F	S^f	TS^F	TS^f	W^F	W^f	Y^F	Y^f	TY^F	TY^f	S^F	S^f	TS^F	TS^f	W^F	W^f	
22	M38	6	♂	1	1	1	1	1	0	3	0	1	0	0	0	0	0	0	0	1	1	0	0	21*
			♀	0	0	4	0	0	1	3	0	1	0	0	0	0	0	0	0	0	0	0	0	
23	M39	5	♂	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	6
			♀	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	
24	M40	5[c]	♂	0	0	2	1	0	0	1	2	1	2	0	0	0	0	0	0	2	0	0	0	22
			♀	0	0	1	0	1	0	3	1	1	1	0	0	1	2	0	0	0	0	0	0	
25	M42	1	♂	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	4
			♀	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
26	M47	5	♂	1	1	2	0	1	1	2	2	0	1	0	0	1	0	0	0	3	2	0	1	37
			♀	0	0	1	0	1	2	2	0	3	0	0	0	3	0	0	0	2	2	3	0	
27	M77	6	♂	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5
			♀	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	
	Total			2	2	14	4	5	4	18	6	7	6	0	0	6	3	0	0	8	5	3	1	94
	Total			4		18		9		24		13		0		9		0		14*		4		95*
	Total			64†										27*										91*†

* Includes 1 TSdr butterfly, not scored for F or sex.
† Excludes butterflies from brood 25.

TABLE 5. VARIOUS TEST-CROSSES

BROOD	MATING	PARENTS ♀	PARENTS ♂		RADIATE (DR)			PLAIN (dr)				Total
					TS^F	TS^f	W^F	TS^F	TS^f	W^F	W^f	
32	M59	1	Trinidad[b]	♂	0	0	0	0	1	0	0	2
		TY^FDr	W^fdr	♀	0	0	0	0	1	0	0	
33	M81	Liverpool	§ 34	♂	0	0	0	0	0	0	0	1
		TS^Fdr	T(−)dr	♀	0	0	0	1	0	0	0	
34	M78	(combined progeny of		♂	1	1	1	1	1	1	2	15*
		18 and 21)		♀	1	1	0	1	0	0	0	

* Includes the following, which could not be scored for F or f:
3 ♂ T(−)dr, 1 ♀ Wdr

Table 6. Various Test-Crosses

BROOD	MATING	PARENTS ♀	PARENTS ♂		DENNIS (Dr) TY^F	TY^f	TS^F	TS^f	W^F	O^f	PLAIN (dr) TY^F	TY^f	TS^F	TS^f	W^F	W^f	Total
35	M55	41	41^f	♂	0	0	0	0	0	0	0	0	0	0	0	1	3*
		WDr	Wdr	♀	0	0	0	0	0	1	0	0	0	0	0	0	
36	M91	49	49	♂	1	1	1	1	0	0	2	1	2	0	0	0	7
		TY^FDr	TS^fDr	♀	0	0	2	1	0	0	1	1	2	1	0	0	
37	M92	52	52	♂	0	0	0	0	1	0	0	0	0	0	0	1	7†
		WDr	W^fDr	♀	0	0	0	0	3	1	0	0	0	0	0	0	
38	M95	52	52	♂	0	1	0	1	0	0	0	0	0	1	0	0	6
		TS^fDr	TY^fDr	♀	0	2	0	1	0	0	0	0	0	0	0	0	

* Includes 1 ♂ Wdr not scored for F or f.

† Includes 1 ♂ WDr not scored for F or f.

gathered into patches separated by red scales in W^F butterflies (Plate-figs. 24-25). The two types are often difficult to score on W butterflies, and in some butterflies they cannot be distinguished because there are so few white scales (columns headed W); TS phenotypes occasionally lack most of their yellow, but F or f can be detected on the underside as marks of a lighter brown than the background color.

Band shape is controlled by a single pair of alleles (*F* and *f*), with broken bands dominant to fused. Thus the F_1 hybrids from the cross $Y^F \times W^f$ (Table 1, broods 5-9) are always TS^F; the backcrosses to Surinam are entirely F (Table 2); and the backcrosses to Trinidad (Table 3) segregate 54F to 78f (or excluding the W phenotypes which are difficult to score, 29F to 33f). The F_2 broods, excluding brood 25, give 59F to 31f, or excluding the W phenotypes, 49F to 24f. The much closer correspondence to the expected ratio in the backcross when W phenotypes are excluded, shows that scoring on these butterflies is unreliable.

Inheritance of yellow line

"Yellow line" denotes a narrow band of scales starting at the base of the forewing and extending roughly along the posterior vein of the cell, towards the band (Plate-fig. 26). The subspecies *H. m. nanna* (with its variant population *H. m. burchelli*) is monomorphic for this phenotype (Plate-fig. 29), but in the present broods its expression is very variable, and it may be represented only by a yellow spot at the base of the wing; when it is combined with the dennis or radiate patterns, this dot is usually all that is visible. All the Surinam parents show the yellow line (in the form of the dot); it is absent in butterflies from Trinidad.

As one has to use a dissection microscope to score this character, only two of each of the backcrosses, one F_2 brood and a small sample from one F_1 have been scored. The butterflies are divided into three classes:

"++" The yellow at the base of the line is solid, with no mixture of black scales; the spot is visible to the unaided eye.

"+" The yellow scales are mixed with black scales, producing a vague yellow spot not immediately apparent to the unaided eye.

"—" The yellow line (or spot) is absent, or if present, the spot is represented by no more than half a dozen yellow scales.

The results (Table 8) show that the yellow line is a character of variable expression, particularly in TS phenotypes, but that it is strongly influenced by the *N* locus, such that N^NN^N butterflies are usually "++", N^BN^B butterflies "—", and heterozygotes variable but often "+". The other factors influencing this character have not been identified, but appear not to include F or sex, which for the sake of simplicity have not been tabulated.

Inheritance of white dots and yellow bar

The "white dots" are a series of faint white markings, comprising all or any of the following (Plate-fig. 27):

(a) a series of up to five white spots on the veins near the tip of the forewing about 3 mms from the edge of the wing;

(b) a white spot, rarely two, near the outer angle of the hindwing;

(c) a series of paired marginal spots at the posterior of the hindwing.

These marks are found only on the underside of the wings. They are not normally found in any of the races of *melpomene* in the Guianas, Venezuela, Trinidad, or Brazil, and I have never noticed them on any wild-caught specimen of any phenotype, although they are, of course, easily overlooked. All these marks are found in the closely related *H. ethilla*, and, all except the white spot on the hindwing, in various subspecies of the closely related *H. elevatus* (Turner, 1967).

TABLE 7. VARIOUS TEST-CROSSES

BROOD	MATING	PARENTS ♀	PARENTS ♂		RADIATE (DR) Y^F	Y^f	TY^F	S^F	S^f	TS^F	TS^f	W^F	DENNIS (Dr) Y^F	TY^F	TY^f	S^F	TS^F	TS^f	W^F	W	W^f	Total
39	M19	1	4	♂	0	0	0	0	0	0	0	0	3	0	0	4	0	0	0	0	0	16
		S^FDr	Y^FDr	♀	0	0	0	0	0	0	0	0	5	0	0	4	0	0	0	0	0	
40	M20	3	1	♂	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	21
		Y^FDr	Y^FDr	♀	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	
41	M24	2	Trinidad	♂	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0	4	0	19
		S^FDr	W^fdr	♀	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	9	0	
42	M54	4	3	♂	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	8
		Y^FDr	Y^FDR	♀	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
43	M63	3	41^f	♂	0	0	0	1	0	1	0	0	0	0	0	1	4	0	0	0	0	9
		Y^FDR	WDr	♀	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
44	M64	3	3	♂	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	26
		Y^FDr	Y^FDr	♀	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	
45	M65	41	2^g	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
		WDr	S^FDr	♀	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
46	M69	9	1	♂	2	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	11
		$T(S^F)DR$	Y^FDr	♀	2	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	
47	M71	2	2^g	♂	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	6
		S^FDr	S^FDr	♀	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	
48	M76	3	3	♂	7	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	25
		Y^FDR*	Y^FDr*	♀	3	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	
49	M85	46	34	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3
		TY^FDr	T(S)dr	♀	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	
50	M86	46	46	♂	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
		Y^FDR	Y^FDR	♀	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
51	M88	34	?	♂	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	4
		TS^FDR		♀	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	
52	M90	46	34	♂	0	0	0	0	0	2	1	1	0	1	0	0	1	2	0	0	2	21
		S^FDR	T(S)dr	♀	0	0	2	0	0	2	1	1	0	0	1	0	0	3	1	0	0	
53	M96	52	52	♂	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	7
		TS^fDR	TS^FDR	♀	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	0	
54	M43	Surinam[a]	Trinidad	♂	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		Y^FDr	W^fdr	♀	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	

* The phenotypes of the parents may have been ♀ Y^FDr, ♂ Y^FDR.

Table 8. Segregation of Yellow Line in a Sample of the Broods

BROOD	YELLOW LINE	PHENOTYPE											
		RADIATE				DENNIS					PLAIN		
		~~Y~~	TY	S	TS	Y	TY	S	TS	W	TY	TS	W
	++	–	–	–	–	–	–	–	9	–	–	–	–
5	+	–	–	–	–	–	–	–	10	–	–	–	–
	–	–	–	–	–	–	–	–	2	–	–	–	–
	++	0	6	0	0	3	11	3	0	–	–	–	–
11	+	0	0	3	2	0	0	9	4	–	–	–	–
	–	0	0	0	0	0	0	0	0	–	–	–	–
	++	6	7	0	0	6	8	0	0	–	–	–	–
13	+	0	0	7	2	0	0	3	0	–	–	–	–
	–	0	0	1	3	0	0	3	5	–	–	–	–
	++	–	–	–	–	–	–	–	0	0	–	0	0
20	+	–	–	–	–	–	–	–	4	0	–	6	1
	–	–	–	–	–	–	–	–	0	8	–	0	8
	++	–	–	–	–	–	–	–	0	0	–	0	0
17	+	–	–	–	–	–	–	–	3	0	–	4	0
	–	–	–	–	–	–	–	–	10	20	–	11	13
	++	–	–	–	–	2	3	0	1	0	4	0	0
26	+	–	–	–	–	0	0	4	4	3	0	8	4
	–	–	–	–	–	0	0	0	0	1	0	1	0

The yellow bar appears to be the same as that found in *H. m. nanna,* extending across the hindwing in the region of the cell (Plate-fig. 29); in these broods it is represented only by a light sprinkling of yellow scales at the inner margin of the hindwing, and another sprinkling, in the form of a faint yellow stripe, beyond the outer end of the cell, or both these marks together (Plate-fig. 28). Usually it is stronger on the underside, but is sometimes visible on the upperside. It has been scored into three grades, "absent" (—), "weak" (+), and "strong" (++).

The white dots and yellow bar appear in a few butterflies in several broods, but only in brood 26 (Table 4), a cross between two TS^{F}Dr butterflies both showing traces of the yellow bar, do these markings appear in a fraction of the brood sufficient for analysis. The segregation of brood 26 for these characters, and for band and dennis, is shown in Table 9: numerical results are extracted in Table 10; sex has no significant influence on the phenotype.

Tabulation of the white dots (absent versus present) against the segregation of the other characters (Table 10), shows that they are little influenced by the segregation of *F, D,* or *B.* But they are strongly influenced by the segregation of *N,* $N^{N}N^{N}$ butterflies invariably showing the dots, the other two genotypes lacking

Table 9. Segregation of Yellow Bar and White Dots in Brood 26

YELLOW BAR	NONE		WEAK						STRONG					
WHITE DOTS	No White Dots		No White Dots		Hindwing Spot + Marginals		All White Dots		Forewing Apicals		Hindwing Spot + Forewing Apicals		All White Dots	
PHENOTYPE	F	f	F	f	F	f	F	f	F	f	F	f	F	f
YD	0	0	0	0	0	0	1	1	0	0	0	0	0	0
TYD	0	0	0	0	1	0	0	0	0	0	1	0	1	0
SD	1	2	1	2	0	0	0	0	0	0	0	0	0	0
TSD	0	0	4	0	0	0	0	0	0	0	0	0	0	0
WD	0	0	3	1	0	0	0	0	0	0	0	0	0	0
TYd	0	0	0	0	0	0	0	0	1	0	3	0	0	0
TSd	3	4	2	0	0	0	0	0	0	0	0	0	0	0
Wd	0	0	2	2	0	0	0	0	0	0	0	0	0	0

them. There is also a strong association between the white dots and the yellow bar.

The association of yellow bar with the segregation of the *N* locus is less clear-cut than that of the white dots, but Table 10 shows that it is considerably influenced by the *N* locus and the *D* locus, the alleles N^N and *D* tending to increase the size of the yellow bar.

The white dots and yellow bar, like the yellow line, are thus in some broods influenced by segregation at the *N* locus, and the yellow bar in addition by the *D* locus; but all these characters are very variable in expression and influenced by other factors. The high frequency of the yellow bar and white dots in one brood only, suggests that at least some of these other factors are genetic.

Linkage

None of the loci *B, D, F, N* is sex-linked, as is plainly shown by segregation in all the large broods. Autosomal linkage can be investigated as follows:

B and *N:* The even segregation in the backcrosses to Surinam (Table 2) and in brood 1 (Table 1) shows that these loci are unlinked, as does the F_2 (Table 4) whose segregation for these loci was discussed above for its fit to a 1 : 3 : 2 : 6 : 1 : 3 ratio. These figures, tested as a 2 × 3 contingency table give $\chi^2_{(2)} = 3.9$, which is not significant.

N and *D:* The backcrosses to Trinidad segregate

	Male parent heterozygous			Female parent heterozygous	
	Dr	dr		Dr	dr
TS	18	21	TS	10	17
W	25	18	W	12	13

(DR included with Dr); the F_2 segregates (excluding brood 25)

	Dr	dr
Y + TY	20	9
S + TS	31	14
W	13	4

None of these segregations shows any sign of linkage.

N and *F:* The backcrosses to Trinidad segregate

	Male parent heterozygous			Female parent heterozygous	
	TS	W		TS	W
F	19	15	F	13	9
f	21	28	f	12	17

and the F_2:

	Y + TY	S + TS	W
F	20	29	10
f	9	15	7

Again there is no sign of linkage.

F and *B:* As *f* does not segregate in the Surinam backcross, nor *b* in that to Trinidad, only the F_2 can detect linkage:

	F	f		F	f
Y+S	7	6	Y+S	7	6
TY+TS+W	52	25	TY+TS	42	18

The test is not very efficient because the genes would be in repulsion, if linked, but the loci appear to be unlinked, for there is a slight excess of "recombinants" over expectation.

D and *B:* Again, only the F_2 tests these loci, and it is not possible to exclude the possibility, described above, that some bbN^BN^B individuals appear the same as BbN^NN^B or BBN^NN^B. The segregation is

Table 10. Association of Yellow Bar (Scored as Absent, Weak, and Strong) and White Dots (Scored as Absent or Present) with Other Characters in Brood 26

	GENOTYPE								
YELLOW BAR	*D–*	*dd*	N^NN^N	N^NN^B	N^BN^B	*B–*	*bb*	*F–*	*ff*
–	3	7	0	10	0	3	7	4	6
+	14	6	3	9	8	5	15	14	6
++	2	4	6	0	0	0	6	6	0

	YELLOW BAR			GENOTYPE								
WHITE DOTS	–	+	++	*D–*	*dd*	N^NN^N	N^NN^B	N^BN^B	*B–*	*bb*	*F–*	*ff*
–	10	17	0	14	13	0	19	8	6	21	16	11
+	0	3	6	5	4	9	0	0	2	7	8	1

	Y + S	TY + TS + W
Dr	13	51
dr	0	27

giving $\chi^2_{(1)} = 6.4$, which is significant at the 2 percent level (one tailed). It therefore seems very likely that *D* and *B* are linked, because no recombinants appear in the F_2. As this is a repulsion F_2, apparently with close linkage, there is little point in estimating recombination.

Brood 46 (Table 7) was set up to test this, an individual of genotype *BbD*^{R}*d* from the F_1 being backcrossed to a Y^{F}Dr Surinamian butterfly. In this cross, as distinct from the other Surinam backcrosses using *BbDd* individuals, both the *D* and *B* loci can be seen segregating. Unfortunately the brood is small, producing 6 *bbD*^{R}*D* parental types, 5 *BbDd* parental types and no recombinants. The probability of getting this result by chance if the genes were unlinked is 2.5×10^{-8} (Fisher's exact test, one tailed), and the 95 percent confidence limit for the combination fraction is 15.5 percent (on the assumption that if one more butterfly had emerged it would have been a recombinant). The loci *B* and *D* are therefore linked, and the recombination fraction between them may be low, especially as the recombinant phenotypes Sdr and Odr are not known in the wild. It will be shown later that recombinations probably do occur.

D and *F:* This is the only relationship which presents difficulties. The F_2 gives no indication of linkage (brood 25 excluded):

	Including W			Excluding W	
	D	d		D	d
F	42	17	F	35	14
f	22	9	f	16	8

Both values of χ^2 (0.0005 and 0.17) are small and not significant. The backcross to Trinidad likewise gives no sign of linkage when the heterozygous parent is male (Table 3, broods 16-18):

	Including W			Excluding W	
	D	d		D	d
F	16	16	F	9	8
f	27	22	f	9	12

On the other hand, when the heterozygous parent is female, we have

	Including W			Excluding W	
	D	d		D	d
F	15	7	F	10	3
f	7	22	f	2	10

Here, $\chi^2_{(1)}$ is respectively 9.89 and 9.08. In addition, brood 52 (Table 7) segregates *D*^{R} and *D*, *F*, and *f*, and although the male parent cannot be scored for F, it is clear that the female is doubly heterozygous *D*^{R}*DFf*. The brood gives

	DR	Dr
F	8	3
f	2	8

for which $\chi^2_{(1)} = 5.84$. All χ^2 values are one-tailed and significant, and both segregations give a recombination fraction of 26% ± 16% (95 percent confidence limit includes 50 percent recombination). It is therefore possible, but not completely proven, that the loci recombine freely in males but are linked in females. The F_2 segregation provides a further check. Using formulae given by Bailey (1961) we can calculate

$$\theta = (1 - r_1)(1 - r_2)$$

where r_1 and r_2 are the recombination fractions in males and females. Taking r_1 as 50 percent, we can estimate r_2 if we know θ:

$$r_2 = 1 - 2\theta$$

Calculating from the data, using Bailey's quadratic formula, $\theta = 26\% \pm 7\%$, and hence $r_2 = 48\%$, with a lower 95 per cent confidence limit of 21 per cent. The F_2 therefore indicates that the recombination fraction in females is 50 percent (no linkage), but leaves open the possibility that it is as low as the 26 percent estimated from the backcross.

The broods therefore lead us to conclude that *D* and *F* are unlinked, but leave open the possibility of moderate linkage or of disturbed segregation in females. It will be remembered that *B* and *D* are linked, and that *F* shows no linkage with *B*, which argues that *D* and *F* are indeed not linked.

Effect of Genetic Background

The band

One male butterfly from brood 5 sired three broods, an F_2 and both types of backcross; the TS phenotypes in these broods therefore enable us to investigate the effect of the gene-complexes of the Surinam and Trinidad subspecies on the expression of the genes affecting the band.

The parental male, a random sample of his sibs, and all TS butterflies from the F_2 and both backcrosses are shown in plate-figs. 30-33. It is clear from this that the loci *D* and *F* (or genes on the same chromosomes) affect the amount of red and yellow in the band, *d* enhancing red and reducing yellow, and *f* enhancing red (its effect on yellow, being its major characteristic, has already been described). Within the TS^{F}Dr phenotypes, butterflies from the Surinam backcross show slightly more yellow and less red than those in the Trinidad backcross, but the difference is very slight and no doubt would not be significant if tested statistically.

There is thus little doubt that the Trinidadian alleles at the *D* and *F* loci (or loci on the same

chromosomes) change TS phenotypes in the direction of the Trinidadian subspecies (less yellow and more red on the upperside), and that the Surinamian alleles have the reverse effect, changing them towards the Surinamian subspecies; other loci may have a similar effect, but in these broods at least it is weaker.

Radiate

The radiate phenotype also seems to be influenced by other loci, as a few butterflies have the hindwing rays expanded into wide wedges which almost touch at their sides (Plate-fig. 34); the rays also appear as prominent red streaks on the underside (normally they are thin and barely noticeable). Many butterflies in the broods in which this variation appeared are now lost, for reasons explained above, so a full analysis is not possible. Of the surviving butterflies, the following show the "spread ray" phenotype:

Broods 21 & 34: 2 out of 6 radiate
Brood 50: 1 out of 5 radiate
Brood 52: 1 out of 2 radiate

As this phenotype does not occur in the pure Belém subspecies (*H. m. thelxiope*) (Text-fig. 1a), nor in the F_1 or Surinam backcrosses, but has appeared in broods 21 and 34, the only Trinidad backcrosses containing radiate, it seems very likely that it results from a gene or genes present in the Trinidadian but not Surinamian stock, and more or less recessive in their effect.

Sex

Plate-figs. 35-36 show the effect of sex on the S^F phenotype in brood 1; in this brood the sexes clearly differ in the amount of yellow. The effect has not been noticed in other broods.

Double Mating

A Surinam female layed 158 eggs between September 28 and November 6; all of those surviving (Table 1, brood 3) were the offspring of her wild radiate mate. Mated accidentally to a Trinidadian male on November 7, she layed one egg on November 8 and then died; this offspring (Table 7, brood 54) is obviously the offspring of her wild mate and not of the Trinidadian male.

Discussion

Identity of the genes

Loci designated by *B, D*, and *N* are described by Turner and Crane (1962) and Sheppard (1963) in breeding experiments with *H. melpomene* from Surinam and Trinidad. It is necessary to show that the loci described here are indeed the same loci, in so far as this can be done without hybridizing the strains used in the various experiments. The *F* locus, althoug it segregated in the broods of the previous authors, was not described by them.

There can be little doubt that the alleles D^R, D and d, and N^N and N^B are the same as those described by Sheppard because phenotypes, dominance, and linkage relations are the same. The only points of difference are that Sheppard did not demonstrate the allelism of D^R and D, and that his genotype N^NN^N was homozygous *BB*; this genotype has not been formed for certain in the present experiments, and its phenotype has been inserted in text-figure 4 (top right) on the strength of Sheppard's results. Turner and Crane describe also the *D* locus (which they attribute to a series of chromosomes *DR*, *Dr*, and *dr*); there can be no doubt that this is the same locus. *N* did not segregate in their broods.

The only locus about which there is any doubt is *B*. Turner and Crane describe a recessive allele *b*, linked to *D* (recombination fraction unknown, although crossovers occurred), which reduces the amount of red in the forewing band. The *b* allele in the present paper is linked in the same way and reduces the amount of red in the band. The difference is that in the experiments of Turner and Crane, the genotype bbN^BN^B had a narrow red band edged with dusky yellow (the TS phenotype), while in the present broods this genotype virtually lacks the band altogether (the O phenotype) (Plate-fig. 20). There are three possible explanations of this:

(a) there are two separate loci, both linked to *D*, which produce this effect;

(b) there are two recessive alleles at the *B* locus, both reducing the red but to a different extent;

(c) the same allele appeared in both experiments, but its effects were altered by genes at other loci.

There is a small amount of evidence in favor of this last hypothesis, in that the amount of red of TS phenotypes is altered by the *D* and *F* loci, and possibly by others (see above). S phenotypes occasionally have small amounts of red in the band, suggesting that other genes can alter the amount of red present. Further, in brood 50, a cross between two Y individuals, a TY phenotype appeared. This may have been an error, or it may indicate the segregation of factors enhancing the red. The *B* locus did not segregate in Sheppard's broods.

Variable expression

In the above broods, the N^NN^N genotype does not always produce the extra yellow and white marks (yellow line, yellow bar, and white spots). To understand this we must remember that none of the genes described in this paper is solely

responsible for the presence or absence of any particular pattern; it only co-operates with a number of other loci in producing its effects. Clearly several segregating loci affect the yellow bar and yellow line, and only when the genetic make-up of the individual is correct at these loci do we find the *N* locus producing and removing the marks. In most broods the genotype at the other loci is such that the markings cannot be produced by the $N^N N^N$ genotype.

The yellow bar sometimes appears in wild *melpomene* in the Guianas; one of these rare individuals is illustrated in Plate-fig. 37.

The same considerations apply to the influence of sex on the S^F phenotype in brood 1.

Recombination

Suomalainen (1965) has shown that chiasmata are absent at meiosis in female moths; if this is so in butterflies, then brood 46 could not have shown recombination, as linkage between loci on the same chromosome would be absolute. The presence of chiasmata in *melpomene* is now being investigated; future breeding experiments must always attempt to measure recombination in both males and females. If there is no recombination in females, it follows that the apparent recombination fraction of 25 percent between *D* and *F* must be spurious.

Conclusions

The polymorphism of *H. melpomene* in the Guianas is controlled by at least four loci, *B, D, F,* and *N,* affecting the distribution of red and yellow marks on the wings, with a further possible locus affecting the amount of red in the band. In addition, substitutions at other loci affect the expression of the four major loci, broadening the rays produced by the allele D^R, causing the allele N^N to produce extra yellow and white marks, and altering the amount of yellow in the band. The loci *B* and *D* are the only ones which are linked, although *D* itself may consist of two linked loci.

Among the homozygous genotypes are bbD^R-$D^R FFN^N N^N$, $bbDDFFN^N N^N$, and $BBddffN^B N^B$, which give the three phenotypes in text-figure 1. It is shown elsewhere (Turner, 1971b) that these are monomorphic subspecies, and that their hybridization accounts for the polymorphism of this species in the Guianas.

Acknowledgments

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The initial breeding experiments, and the preparation for fieldwork in Surinam were conducted at the William Beebe Station for Tropical Research, Trinidad, which was then operated by the New York Zoological Society, under the kind hospitality of the then Director, Jocelyn Crane Griffin, who was responsible for the guidance of research on the Heliconiinae. Breeding experiments in Liverpool would not have been possible but for generous allocations of greenhouse space by Professor C. A. Clarke, FRS, and Professor P. M. Sheppard, FRS; Professor Sheppard cared for, and set up, the broods after I had left Liverpool.

The considerable work of breeding the butterflies in Trinidad was assisted by Thomasina Lai-Fook, Alleen Lai-Fook, and Jogie Ramlal. Transportation of the stocks was made possible by the cooperation of the staff of British West Indian Airways and the British Overseas Airways Corporation.

Fieldwork in Surinam was much helped by contact with Dr. D. C. Geijskes of the Suriname Museum, Drs. E. H. Jonkers of the Netherlands Economic Mission, and various officers of Suralco (Surinam Aluminium Company), who operate the road to Brokopondo.

Analysis of the results was in part carried out under a Nature Conservancy Studentship in the laboratory of Professor E. B. Ford, FRS, whose interest in this work, together with that of Professor Sheppard, has always been a great encouragement to me.

I am grateful to Dr. A. H. D. Brown and Dr. K. S. Brown, Jr. for their helpful criticism of the script.

Summary

1. *Heliconius melpomene* from Trinidad, West Indies, were crossed with *melpomene* from a slightly polymorphic population belonging to another subspecies from central Surinam.

2. The presence or absence of red marks at the base of the wings is controlled by three alleles at a single locus (*D*).

3. The amount of red and yellow in the band on the forewings is controlled by the interaction of two loci (*N* and *B*).

4. The distribution of yellow pigment in the band is controlled by a single locus (*F*).

5. *B* and *D* are linked at not more than 16 percent recombination, and the other loci are unlinked, although *F* and *D* show some irregular segregation.

6. Three characters not found in the parent stocks appeared in some of the hybrids; these

were controlled chiefly by the *N* locus. The characters are normally found in various close relatives of *H. melpomene*.

7. There may be an additional locus enhancing the amount of red on the forewing.

8. The amount of red and yellow in the band is influenced by the *D* and *F* loci, and further loci cause the ray marks on the hindwings to fuse together when they are backcrossed into Trinidadian stock.

9. The three Guianian subspecies of *melpomene* are probably homozygous in different ways for alleles at the four major loci, the Surinam and Trinidad races differing by one substitution at each locus.

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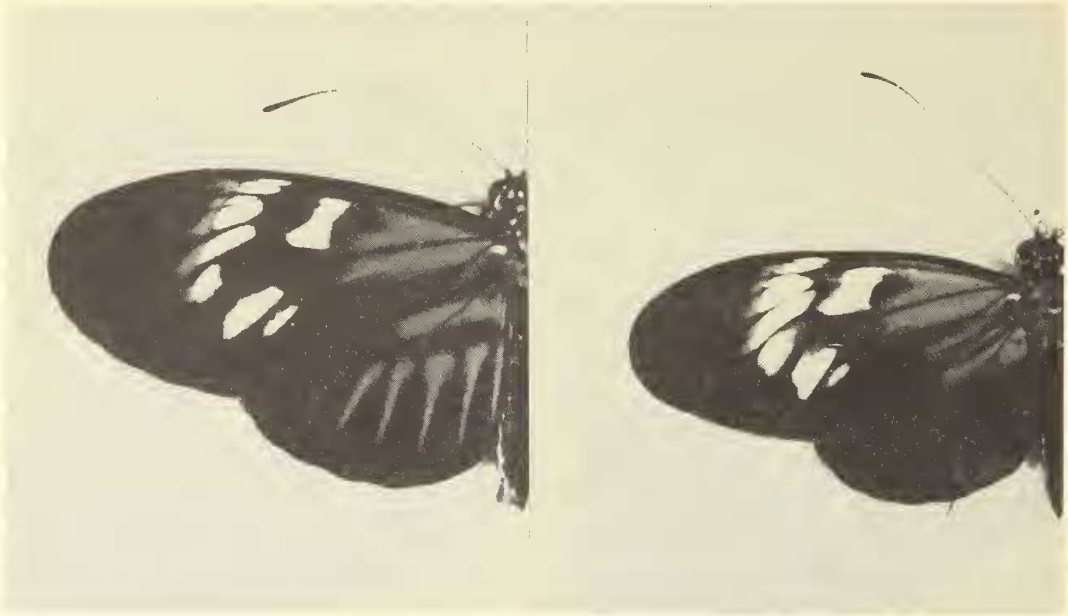
EXPLANATION OF THE PLATES

1 2

3 4

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 1-2 Y^F
Figs. 3-4 Y^f



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FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 5-7 TY^F

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FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 8-9 TY^{f}
Figs. 10-11 S^{F}

12

13

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 12-13 S^f

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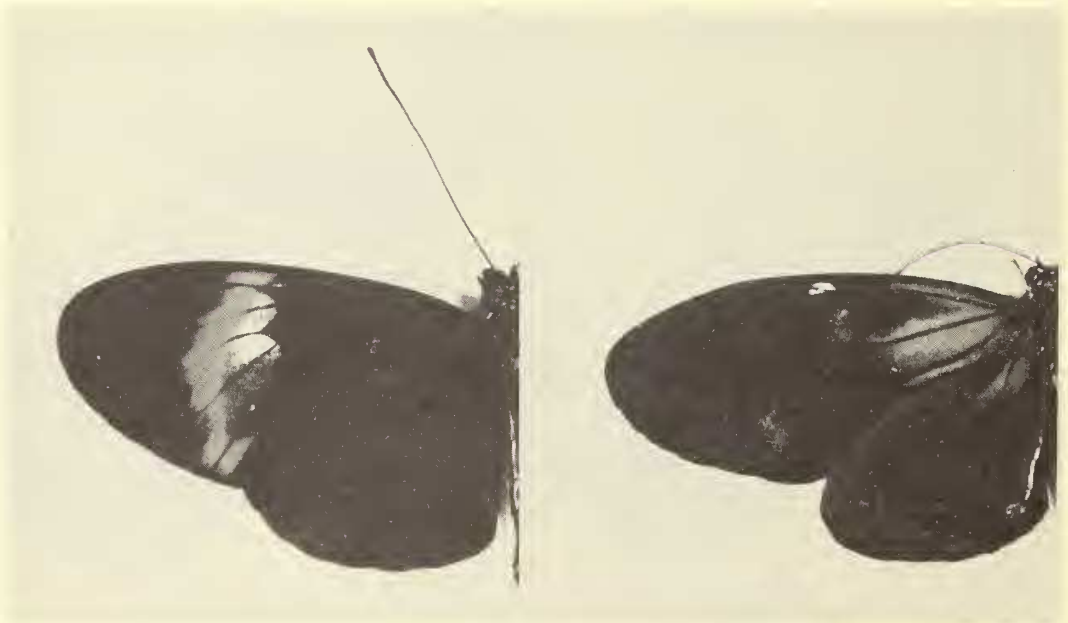
FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 14-16 TS^{F}

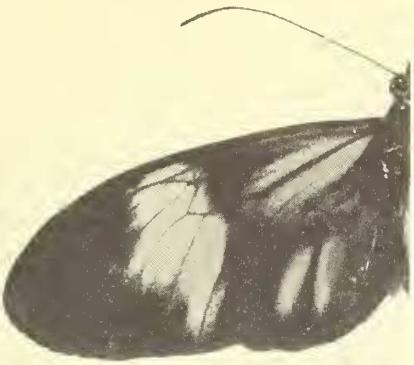
17 18



19 20

FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 17-19 TS^f
Fig. 20 O^f



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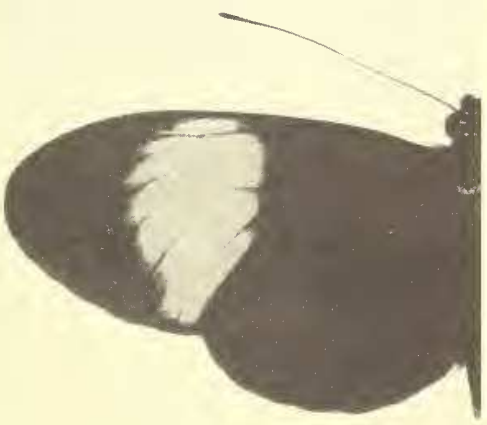

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FIGURES 1-23. The main phenotypes obtained in the broods, showing the radiate, dennis, and plain patterns in combination with the various band-types, as follows:

Figs. 21-23 W

24

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FIGURES 24-25. The effect of the F locus on the distribution of white scales on the underside of the W phenotype.

Fig. 24 W^F
Fig. 25 W^f

FIGURE 26. The yellow line pattern

FIGURE 27. The white dots

FIGURE 28. The yellow bar

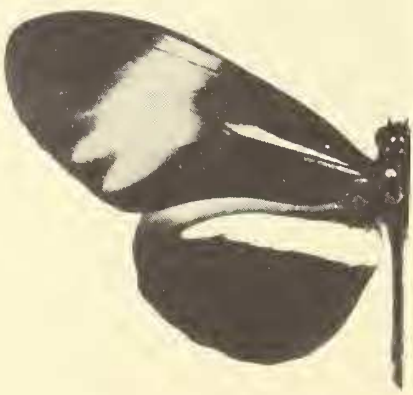
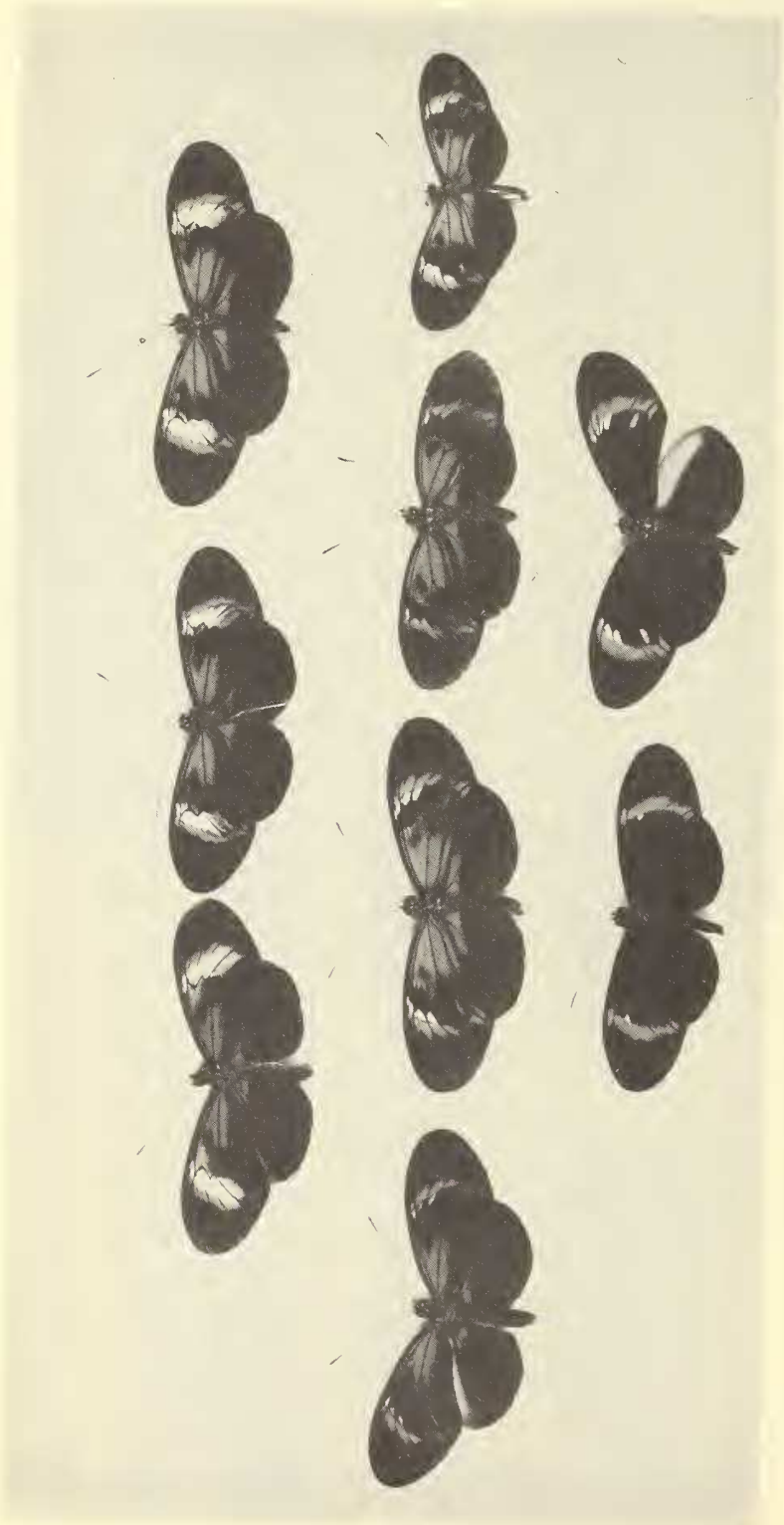


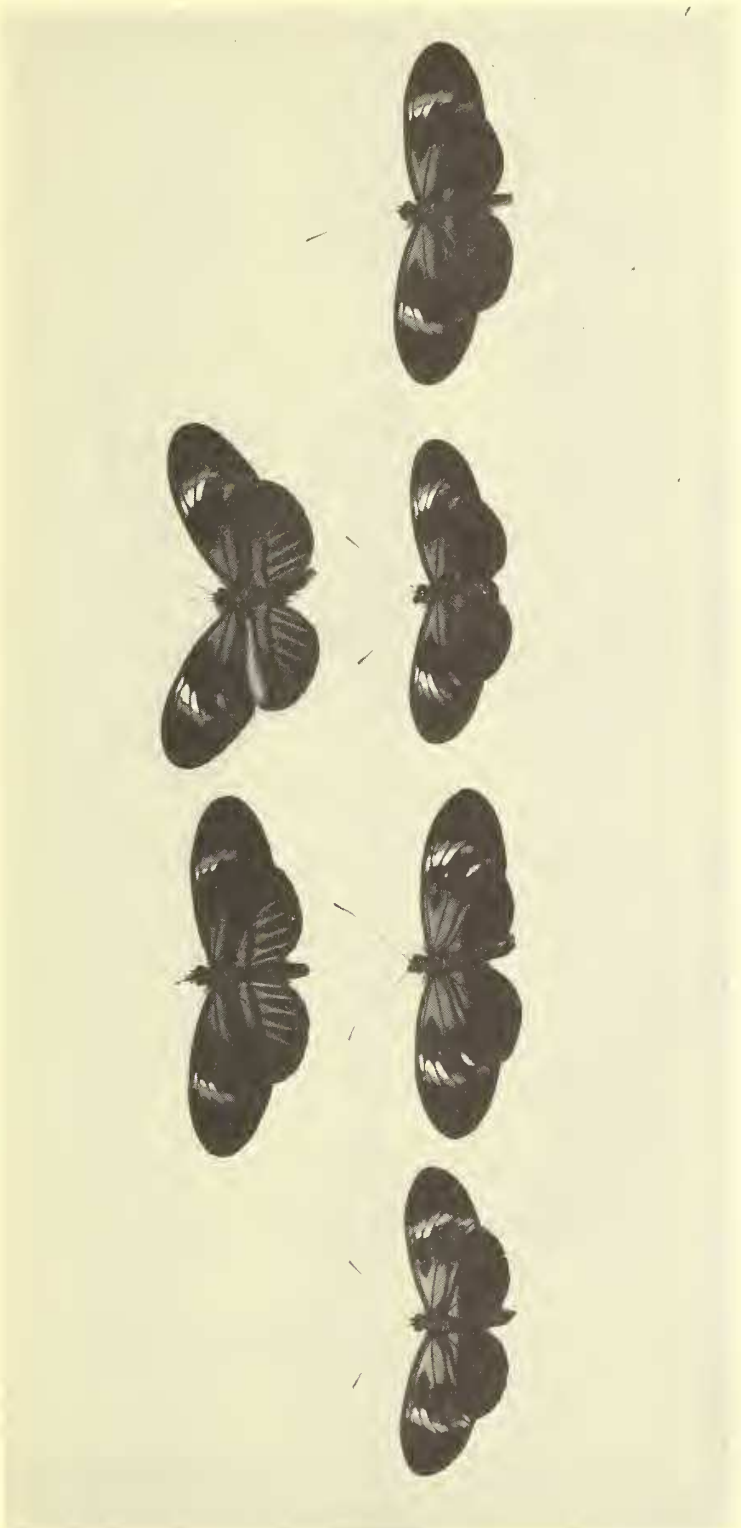
FIGURE 29. The Brazilian subspecies *H. melpomene nanna*

FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male

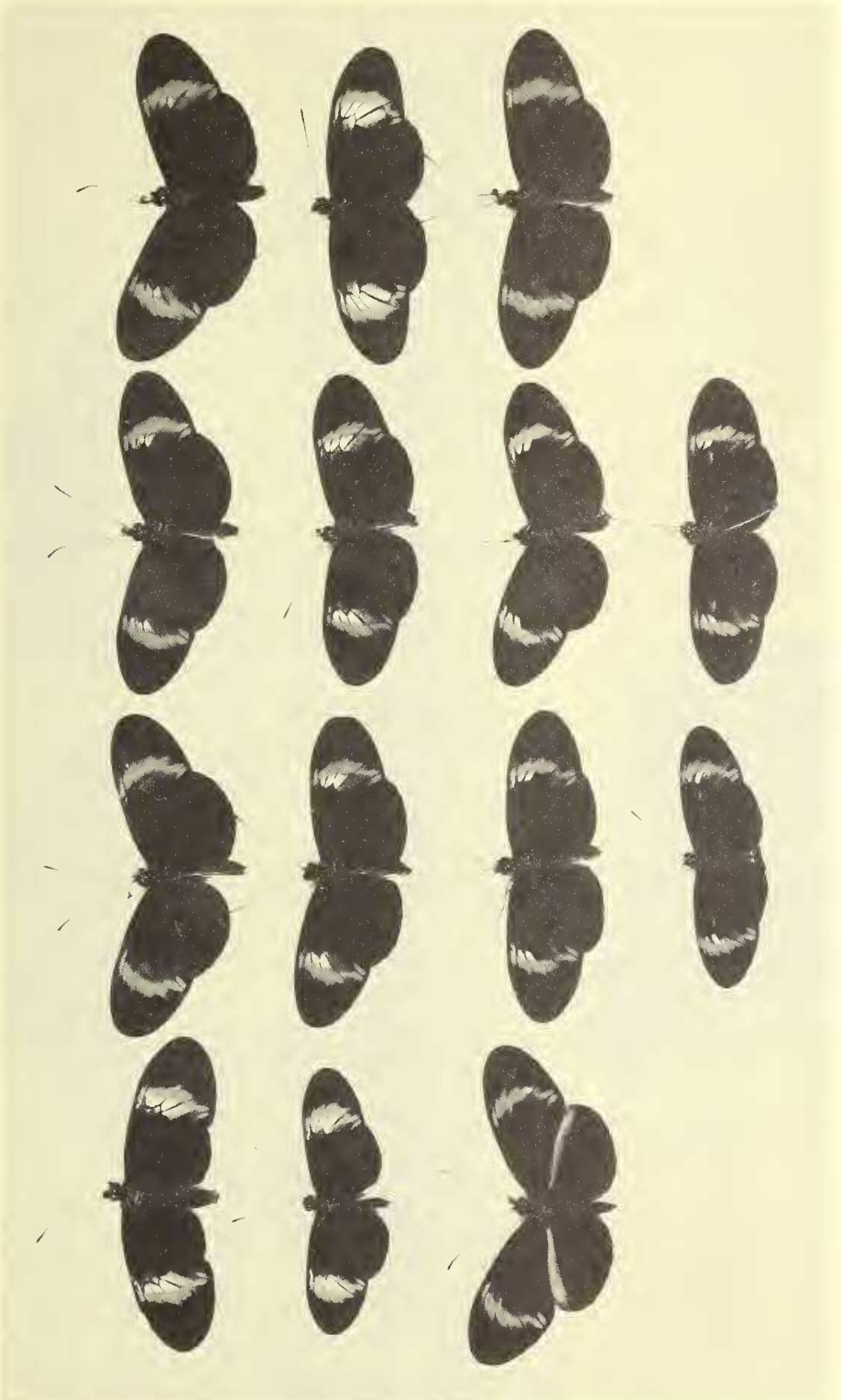
Fig. 30 The male F_1 parent and a few of his sibs (brood 5)



FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male
Fig. 31 The F_2 (brood 24)



FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male
Fig. 32 The backcross to Surinam (brood 11)



FIGURES 30-33. Variation in the yellow marks in TS phenotypes sired by the same male
Fig. 33 The backcross to Trinidad (brood 17)

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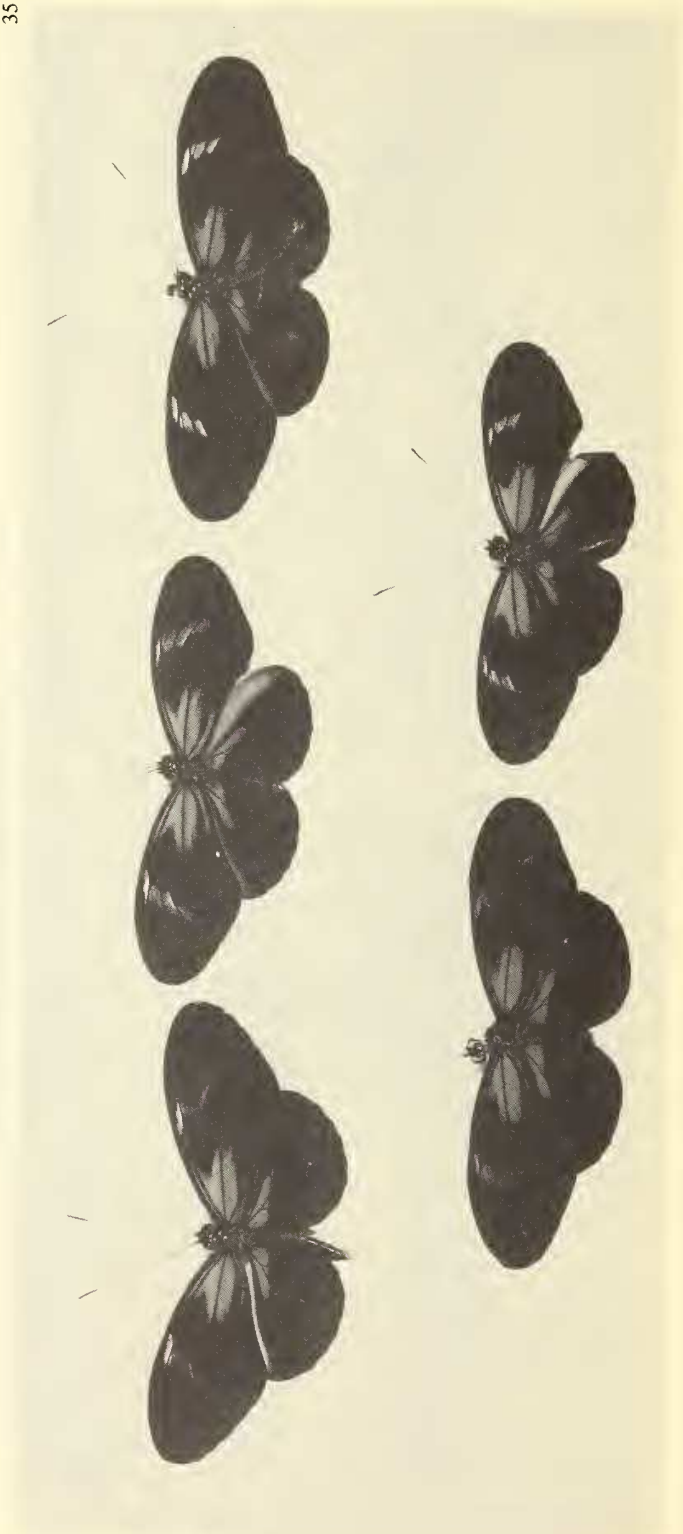
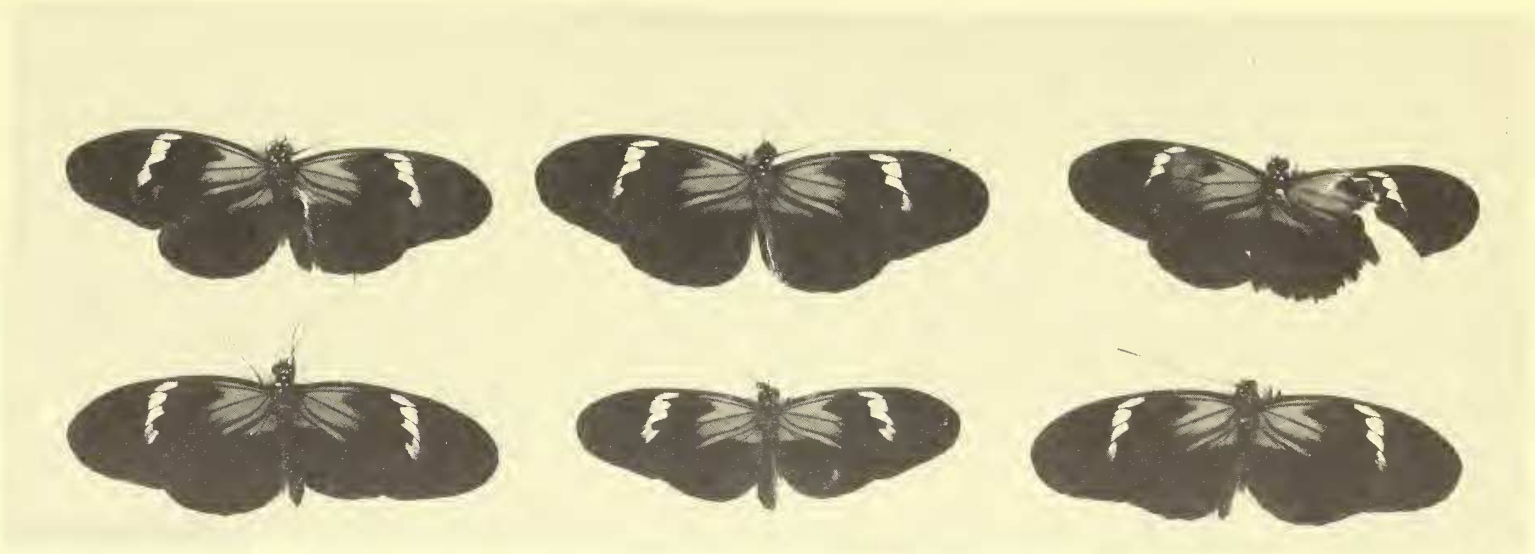


FIGURE 34. A phenotype with the ray marks partly fused together

FIGURE 35. Male S^F phenotypes in brood 1



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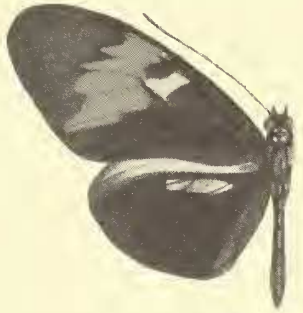

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FIGURE 36. Female S^F phenotypes in brood 1

FIGURE 37. The type figure of var. *eltringhami* J & K (by permission of the Royal Entomological Society of London) (from Joicey and Kaye, 1917).